Diabetic Foot Infections: Current Diagnosis and Treatment

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Abstract:

Diabetes mellitus (DM) is a global epidemic, and diabetic foot ulcer (DFU) is one of its most serious and costly complications. DFUs result from a complex interaction of a number of risk factors. Once the protective layer of skin is broken, deep tissues are exposed to bacterial infection that progresses rapidly. Patients with DFUs frequently require amputations of the lower limbs and, in more than half the cases, infection is the preponderant factor. Given the challenges of treating these complex infections, this paper aims to provide a hospital-based framework for the diagnosis and treatment of diabetic foot infections (DFIs). We propose a treatment-oriented assessment of DFIs based on a cross-examination of the medical, foot, and wound history; a systemized and detailed physical examination; and the results of complementary diagnostic procedures. We stress the need for a clinical diagnosis of DFIs and the importance of microbiological evaluation for antibiotic therapy guidance. Regarding treatment, we propose a multidisciplinary approach prioritizing invasive infection drainage, necrosis debridement, and the prompt start of empirical antibiotic therapy, followed by complete and appropriate vascular reconstruction. For severe DFIs, we suggest that negative pressure wound therapy (NPWT) be included in the treatment pathway. We also provide rules for managing particular situations, such as osteomyelitis. It is our hope that this protocol will improve the hospital management of DFIs and, ultimately, the prognosis of DFI patients.

Key words: Diabetic foot infections, osteomyelitis, diabetic foot ulcers

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List of Abbreviations

AGE	– advanced glycation end-products	ESR	– erythrocyte sedimentation rate	PAD	– peripheral arterial disease
bFGF	– basic fibroblast growth factor	HBOT	– hyperbaric oxygen therapy	PDGF	– platelet-derived growth factor
CRP	– C-reactive protein	IL-6	– interleukin-6	TcPO2	– transcutaneous oxygen pressure
CT	– computed tomography	KGF-2	– keratinocyte growth factor-2	TGF-ß	– transforming growth factor-ß
DFI	– diabetic foot infection	NO	– nitric oxide	TIMP	– tissue inhibitor of metalloproteinases
DFU	– diabetic foot ulcer	MMP	– matrix metalloproteinases	VEGF	– vascular endothelial growth factor
DM	– diabetes mellitus	MR	– magnetic resonance		
ECM	– extracellular matrix	NPWT	– negative pressure wound therapy		

Introduction

The world is facing a major epidemic of diabetes mellitus (DM). There are an estimated 171 million diabetic patients worldwide and this number is expected to double by the year 2030 [1]. All of these patients are at risk for developing a diabetic foot ulcer (DFU). A DFU is any full-thickness wound below the ankle in a diabetic patient, irrespective of duration. Based on current studies, the annual population-based incidence is 1 to 4% with a prevalence of 4 to 10%, and the estimated lifetime risk is 25% [2]. According to a study published by the Eurodiale study group [3], approximately 58% of DFU patients will become clinically infected. Patients with DM frequently require minor or major amputations of the lower limbs (15 to 27%) and in more than 50% of cases, infection is the preponderant factor [4]. Major amputation is associated with significant morbidity and mortality (ranging from 13 to 40% at 1 year to 39 to 80% at 5 years 2) in addition to immense social, psychological, and financial consequences [5].

Diabetic foot infection (DFI) treatment accounts for up to one-quarter of all diabetic admissions in both Europe and the United States making it the single most common reason for DM-related hospital admission [6]. In the longer term, costs are even higher as DFUs have recurrence rates of up to 70% in centers of excellence, resulting in repeated interventions and progressive disability[7].

Recognizing this predictable progression, the solution includes developing structured screening tools to identify those at risk and implementing both standardized education and prevention protocols. Different countries and healthcare systems have implemented such approaches to diabetic foot care, some with reported success [4] and others with reported failure [8]. As stated by Lavery et al. [9], however, even with the best of preventive care, 9% of patients with DM will still develop a DFI, with the consequent risk of amputation. This partial failure of prevention strategies supports the need to develop a framework for diagnosing and treating patients with suspected DFIs. This assumes special importance in DFIs that require hospitalization, as strategies have proven efficacy in reducing morbidity, mortality, psychological distress, and financial costs[10]. Despite the publication of different clinical guidelines for DFI management, there is still practical variation in inpatient management [10,11]. This paper aims to provide a hospital-based framework for the diagnosis and treatment of DFIs, based on a pathophysiological approach.

Pathophysiology

A prior DFU is an almost obligatory prerequisite for DFIs. This is true even though, in some cases, the wound may have closed over before DFI presentation [9].Numerous observational studies have indicated that DFUs have a multifactorial nature. It is well established that insulin deficiency (absolute or relative) is the basis of the biochemical abnormalities that lead to the organic complications of diabetes mellitus [12] (namely, neuropathy) and the biological deficits of tissue healing and regeneration. It has also been established that perfect and persistent glycemic control, with either insulin or oral agents, stop [13] and probably regress [14] these complications. DFUs result from a complex interaction of two major risk factors: neuropathy and peripheral vascular disease. Neuropathy, both symmetric and bilateral, plays the main role with varying degrees of alterations in autonomic, sensory, and motor functions. Playing a secondary role is peripheral vascular disease resulting from atherosclerosis **(Figure 1)**. Approximately 50 to 60% of all DFUs can be classified as neuropathic. Signs or symptoms of vascular compromise are observed in 40 to 50% of all patients with the vast majority having neuroischemic ulcers, and only a minority of patients have purely ischemic ulcers [15].

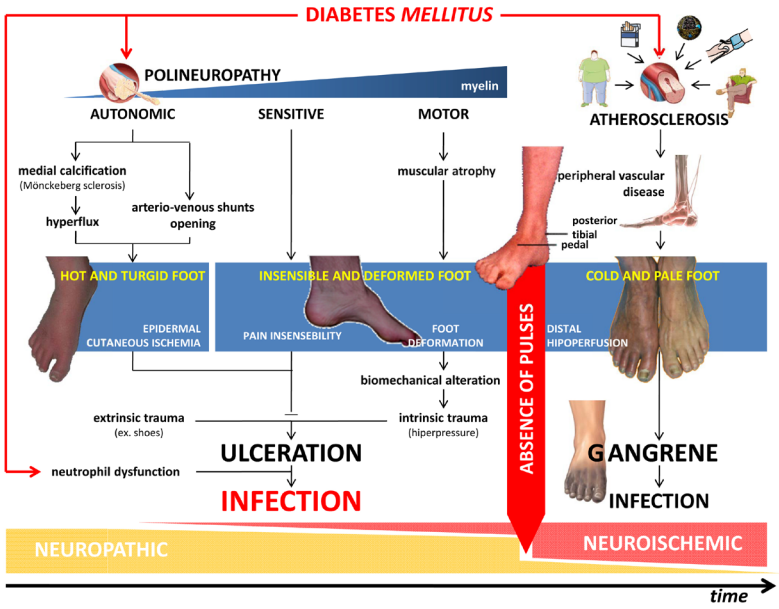

Figure 1: Diabetic foot ulcer (DFU) and diabetic foot infection (DFI) pathophysiology. DFU results from a complex interaction of a number of risk factors. Neuropathy (with alterations in motor, sensation, and autonomic functions) plays the central role and causes ulcerations due to trauma or excessive pressure in a deformed foot without protective sensibility. Once the protective layer of skin is broken, deep tissues are exposed to bacterial colonization. Infection is facilitated by DM-related immunological deficits, especially in terms of neutrophils, and rapidly progresses to the deep tissues.

Neuropathy

Diabetic peripheral neuropathy results from degenerative changes of axons and affects all nerve fibers, but at different times. The non-myelinated autonomic nerve fibers are affected first, resulting in autosympathectomy with consequent medial artery calcification (Mönckeberg calcification), microvascular thermoregulatory dysfunction, and arteriovenous shunting [16]. Mönckeberg calcification, unlike atherosclerosis, does not reduce the arterial internal diameter. Noninvasive flow studies [17] have demonstrated a hyperperfusion of the foot, especially of the deep tissues, while transcutaneous oxygen pressure (TcPO2) measurements have shown a relative epidermal ischemia as a result of the microvascular dysfunction and arteriovenous shunting [18]. Autonomic neuropathy can cause anhidrosis leading to dry skin, cracking, and fissuring. This can create a portal of entry for bacteria [19]. Contrary to the classical conceptualization, it is of the utmost importance to recognize that most diabetic persons have adequate circulation necessary for a cure [17]. In this time frame, in which autonomic dysfunction dominates, there is clinically a pathophysiologically resultant hot and turgid foot. Shortly after, other forms of neuropathy become superimposed. Sensory neuropathy begins with poorly tolerated tactile allodynia and thermal hyperalgesia. As progressively thicker myelinated fibers are affected this progresses to an objective loss of sensation and proprioceptive dysfunction [20]. Motor neuropathy results from the axonal degeneration of the large motor myelinated fibers. This causes anterior crural muscle atrophy or intrinsic muscle wasting, which leads to foot deformities and consequent altered foot biomechanics with foot pressure redistribution [19]. As the disease progresses, the foot becomes clinically insensitive and possibly deformed (claw toes, hammertoes, prominent metatarsal heads, etc.).

Peripheral Arterial Disease (PAD)

The first notion to consider is that vascular disease of the diabetic foot always results from tight and obliterative atherosclerosis in the large limb vessels and not from, as is most commonly believed, microvascular disease [21]. This confusion results from the theoretical conclusion that diabetic multifactorial microvascular insufficiency applies to the foot; however, even the almost universal basal membrane thickening is not likely to be present in the foot capillaries [22]. DM is associated with a near 3-fold increased risk of accelerated atherosclerosis, which is histologically identical to that seen in the non-diabetic population [23]. This underlines the importance of identifying and aggressively managing associated vascular risk factors, such as obesity, cigarette smoking, dyslipidemia, hypertension, and sedentary behavior [24]. The major difference in the diabetic population is the distribution of disease, which tends to be symmetrical with a more distal (tibio-peroneal) involvement and a predominance of long segment occlusions and calcification [25]. When femoral disease is present, it tends to be diffuse with no single dominant focal lesion [23].

Ulceration

Ulceration of the diabetic foot, either neuropathic or ischemic, does not occur spontaneously. It usually follows some form of extrinsic or intrinsic trauma[26]. While extrinsic trauma may include any kind of thermal (e.g., scalding from hot water), chemical (e.g., abrasion from callus treatment solutions), or localized mechanical (e.g., puncture wounds from foreign objects) injuries, the most common injury leading to ulceration is continuous low-pressure trauma, typically from ill-fitting shoes, and injuries due to chronic repetitive trauma from walking or day-to-day activity [27]. Intrinsic traumas are also easily understood as they result from foot deformities (foot drop, equinus, hammertoes, and prominent plantar metatarsal heads) and consequent altered foot biomechanics [4]. These foot

deformities result from the atrophy induced by motor neuropathy of the foot's intrinsic muscles.

Infection

Once the protective layer of skin is broken, the deep tissues are exposed to bacterial colonization [28]. Qualitative and quantitative aspects of wound microbiology are critical determinants of the wound outcome (Figure 2).

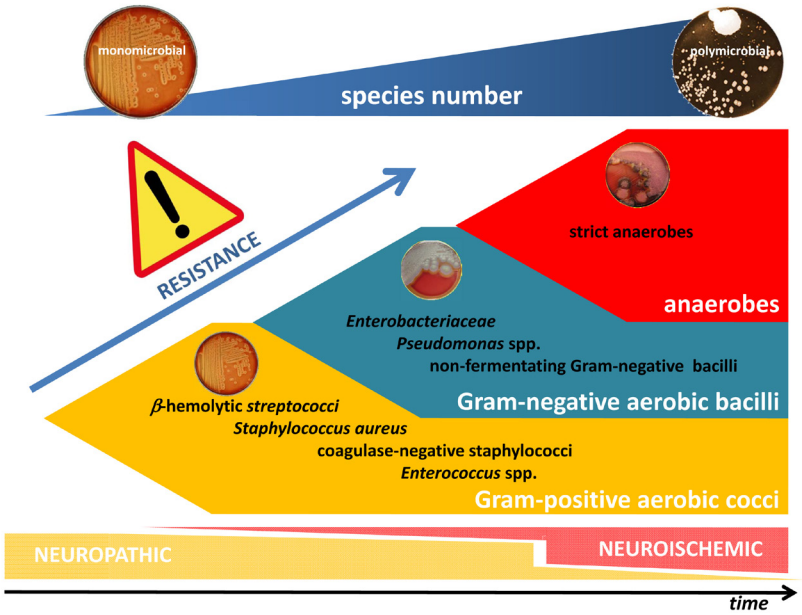

Figure 2: Qualitative and quantitative aspects of diabetic foot infections (DFIs). Staphylococcus aureus and ß-hemolytic streptococci are the first microorganisms to colonize and acutely infect breaks in the skin. Chronic wounds develop a more complex polymicrobial microbiota, including aerobic Gram-negative rods and anaerobes.

Staphylococcus aureus and ß-hemolytic streptococci are the first microorganisms to colonize and acutely infect breaks in the skin. Chronic wounds develop a more complex polymicrobial microbiology, including aerobic Gram-negative rods and anaerobes. Gram-negative bacilli, mainly Enterobacteriaceae, are found in many patients with chronic or previously treated infections, and Pseudomonas aeruginosa is specifically associated with wounds treated with wet dressings [29]. Less virulent bacteria such as Enterococcus spp., coagulase-negative Staphylococcus spp., or Corynebacterium spp. may also represent true pathogens [29]. Anaerobes are rarely the sole pathogen, but they often participate in a mixed infection with aerobes, especially in cases of deep tissue infection [30]. These mixed infections provide an optimal opportunity for microbial synergy, which increases the net pathogenic effect and hence the severity of infection [28]. Accordingly, the composition of the polymicrobial wound flora is likely to be more important than the presence of specific pathogens.

Assuming that the qualitative microbiology remains constant, the probability of wound infection increases as the microbial load increases to a critical level. At this level, infection or a failure to heal is considered almost inevitable [28]. In complex extremity wounds, this critical level has been established by Breidenbach et al. [31] as a bacterial tissue count $\geq 10^4$ CFU/g. There are exceptions to this rule of thumb, however, as various organisms have different intrinsic virulence potentials. A good example is ß-hemolytic streptococci which is able to induce tissue damage at 10^2 CFU per gram of tissue, while greater counts of less pathogenic organisms may be of little clinical significance [32]. A third critical factor is the efficacy of the host's immune response in dealing with wound microflora. In DFUs, infection is facilitated by intrinsic immunological deficits, especially in terms of neutrophil dysfunction. [33] Notwithstanding, infection is also facilitated by local potentiating factors such as tissue necrosis, hypoxia (due to poor local perfusion accentuated by the hypermetabolic state and microbial cellular metabolism), ischemia, and the particular anatomy of the foot (i.e., it is divided into several compartments, explaining the rapid spread of infection), all of which impair immune cell activity in the wound environment [10].

All of these complex interactions have been systematized by the wound infection continuum [34]. This concept describes the effects of increasing bacteria quantity and diversity in

wound tissue, and their relationship to the quality of the host's immune response. Simplistically, in relation to infection, the outcome of a wound may be depicted as the result of an equation:

$$\text{outcome} = \frac{(\text{inoculum x virulence}) + \text{potentiating factors}}{\text{host resistance}}$$

Acute and Chronic Wound Healing Physiology

In the acute wound setting, once the protective barrier is broken, the physiologic process of healing is immediately set in motion **(Figure 3)**.

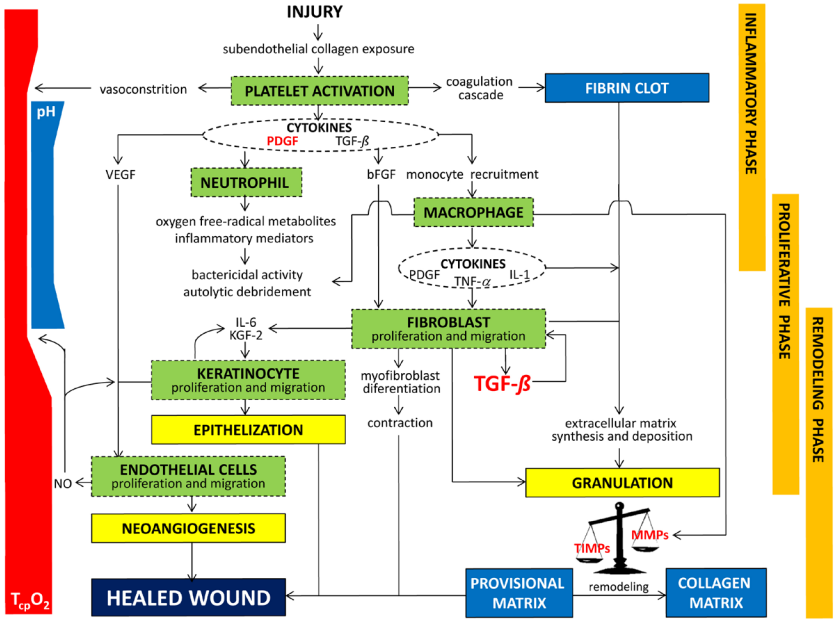

Figure 3: Model of acute wound healing. Wound healing is divided into three sequential but overlapping phases: (1) the inflammatory phase, (2) the proliferative phase (re-epithelialization, granulation, and neoangiogenesis), and (3) the remodeling phase (extracellular matrix remodeling). This complexly orchestrated biochemical cascade is characterized by signature events and cells, and their molecular regulators.

In this classic model [35] wound healing is divided into three sequential but overlapping phases: (1) the inflammatory phase, (2) the proliferative phase (re-epithelialization, granulation, and neo-angiogenesis), and (3) the remodeling phase (extracellular matrix remodeling). This complexly orchestrated biochemical cascade is characterized by signature events and cells, and their molecular regulators [36]. In recent years, the scientific study of wound healing has progressed greatly making it impossible to summarize all the current knowledge in this article. Consequently, we will only describe the process of wound healing to the degree necessary for the clinician to apply this basic science to selecting treatment interventions and understanding their expected outcomes.

The inflammatory phase starts with injury-related subendothelial collagen-mediated platelet activation. Platelets degranulate, releasing proinflammatory and proliferative growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß), and basic fibroblast growth factor (bFGF). This initially results in vasoconstriction and the formation of a fibrin clot, which becomes the pathway for cellular influx and the primary foundation for collagen deposition [37]. Vasoconstriction also promotes a hypoxic and acidotic wound space (secondary to anaerobic metabolism), which stimulates vascular endothelial growth factor (VEGF) release and fibroblast infiltration [38]. In the second stage of the inflammatory phase, leukocytes attracted by chemotaxis, supplant platelets as the dominant cell type. Neutrophils are the first to begin bactericidal activities, using inflammatory mediators and oxygen free-radical metabolites. As these begin to wane, circulating monocytes, attracted by TGF-ß and PDGF, enter the wound and mature into tissue macrophages. These cells debride the wound on a microscopic level and produce cytokines necessary for the proliferative stage [36].

The proliferation phase begins at 72 hours, as recruited fibroblasts migrate inward from the wound margins over the fibrin matrix established during the inflammatory phase. Initially stimulated by bFGF and PDGF, fibroblasts begin to synthesize and deposit extracellular matrix (ECM) components (the provisional matrix). ECM is composed of fibrinous elements

(collagen, elastin and structural glycoproteins) and glycosaminoglycans (chondroitin sulfate, hyaluronic acid and dermatan sulfate), which attract large amounts of water and sodium collagen. Soon, fibroblasts become the dominant cell type and self-express TGF-ß – the negative regulator of acquired and adaptive immunity and the central regulator of tissue repair – directing collagen matrix formation and transforming into myofibroblasts, whose activities incite wound contraction and significantly reduce the area to be filled. This new microenvironment, with ECM-embedded fibroblasts coated with a layer of fibronectin, constitutes the scaffolding for the subsequent neo-angiogenesis and re-epithelization. Initially directed by fibroblasts that express keratinocyte growth factor-2 (KGF-2) and interleukin-6 (IL-6), and later by nitric oxide (NO) and IL-6 self-expression, keratinocytes at the wound edges migrate laterally along the basal membrane and both proliferate and differentiate to produce the epidermis [36]. In turn, keratinocytes direct neo-angiogenesis at the wound edge by expressing VEGF, which is upregulated by NO. The vasodilation induced by NO also aids in the movement of inflammatory cells to the site of injury.

The final stage is the remodeling phase. This phase most clearly shows the overlapping of all woundhealing phases. Although classically described at the end of the proliferative phase, it actually begins concurrently with the formation of the granulation tissue and continues until the tissue reaches maturation. This phase involves a delicate equilibrium between tissue deposition and degradation, controlled by the activity of proteolytic enzymes – mainly matrix metalloproteinases (MMPs) – and their natural inhibitors – tissue inhibitors of metalloproteinase (TIMPs). Collagenase and other MMPs degrade Type III collagen, and Type I collagen fibrils are then laid down parallel to the wound lines of tension in an organized fashion, with strong cross-linking and bundle construction, replacing the lost tissue with an increased tensile strength [35].

Chronic wounds are wounds that, following the orderly and timely repair process, fail to establish a sustained anatomic and functional result [39]. The current thinking is that imbalances exist in the molecular environment of these chronic nonhealing wounds. That is, when the scales are tipped towards high levels of MMPs and proinflammatory cytokines along with senescent cells, there is a low mitogenic activity that invariably results in chronicity [40]. In diabetic wounds, a persistent inflammatory phase is commonly witnessed at histopathology, which is associated with a delay in the formation of mature granulation tissue and a reduction in wound tensile strength [41]. Continuous bacterial infection [42] and increased advanced glycation end-product (AGE) formation [43] limit the cytokine (mainly TGF-ß)-mediated switch to the later granulation tissue formation phase. This results in prolonged inflammation and increased neutrophil infiltration with consequent protease activity.

Assessment

Recognizing important risk factors and making a logical treatment-oriented assessment of DFIs requires a consistent and thorough diagnostic approach. Such an evaluation involves the careful assimilation of global medical, foot, and wound history; a systemized and detailed physical examination; and the results of complementary diagnostic procedures. Various systems have been proposed to classify DFUs, but none have gained widespread acceptance. The International Working Group of the Diabetic Foot developed the PEDIS classification system [44], which presents internationally applicable guidelines that can reliably predict the outcome of diabetic foot management [45].

The PEDIS system classifies all DFUs into subcategories of five main parameters (Perfusion, Extent/size, Depth/tissue loss, Infection and Sensation) according to strict criteria. Although it was not developed as a guide for daily management or to predict the outcome of an individual patient, it considers all the potentially useful information obtained from the clinical history, foot examination and diagnostic exams. Consequently, the use of this systematic examination ensures that important aspects are not overlooked.

Vascular Examination

The vascular examination ("perfusion" in the PEDIS system) begins with the clinical evaluation of intermittent claudication symptoms and the complementary palpation of dorsal pedal and posterior tibial pulses (**Figure 4**).

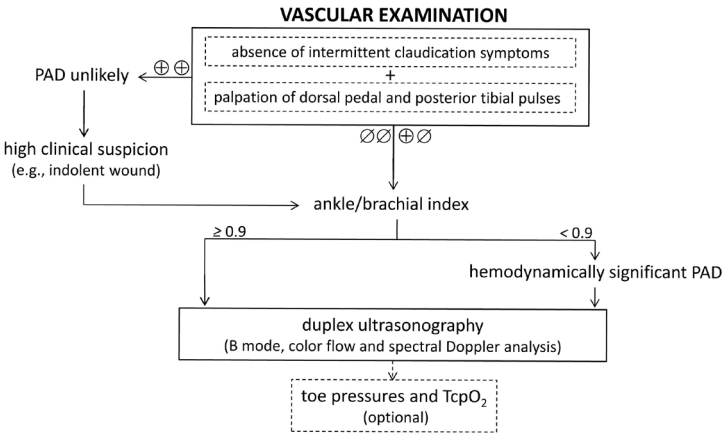

Figure 4: Vascular examination. The presence of both pulses in the foot, in combination with the absence of intermittent claudication, renders significant PAD unlikely. If one or two pulses are absent, clinically relevant PAD is more likely. Establishing the ankle/brachial index (by dividing the ankle pressure by the Doppler pressure measured in the brachial artery) is the next step, but the usefulness of this technique in diabetic patients is limited. Thus, in clinical practice, the formal revascularization decision is based on information from previous clinical tests and duplex ultrasonography (B mode, color flow and spectral Doppler analysis). The complexity and cost of toe pressure or TcpO2 use precludes their generalized application outside clinical studies.

Based on the present literature [25,44], the presence of both pulses in the foot, in combination with the absence of intermittent claudication, renders significant PAD unlikely. On the contrary, if one or two pulses are absent, clinically relevant PAD is more likely; however, pulses can be absent because of edema, making additional objective vascular assessment necessary to exclude PAD or to grade it if it is present [46]. In non-diabetic patients, measuring the systolic ankle blood pressure with a hand-held Doppler device and calculating the ankle/brachial index (by dividing the ankle pressure by the Doppler pressure measured in the brachial artery) is the next step [25]. An index <0.9 confirms a hemodynamically significant occlusive disease, and progressive decrements constitute a rough estimate of the severity of the occlusive disease in non-diabetic patients. Unfortunately, because of the arterial media calcification observed in up to one-third of diabetic patients, this technique has limited usefulness in this special population [47]. On the other hand, more complex techniques, such as systolic toe-pressure measurement or TcPO2, were better predictors of healing in several studies [25].

Although the PEDIS classification system suggests the use of toe pressures or TcPO2 to exclude clinically relevant vascular disease [44], the complexity and cost of its use precludes its generalized application outside of clinical studies. When used in clinical practice, the formal revascularization decision is based on information from previous clinical tests and duplex ultrasonography (B mode, color flow, and spectral Doppler analysis) [48]. The ultrasonographic exam is a good, noninvasive method for delineating the peripheral arteries and enables the distinction of high-flow functional arteriopathy. If a possible revascularization is considered, intra-arterial digital subtraction angiography is conducted to properly visualize the arteries of the feet and evaluate the feasibility of revascularization [49]. Magnetic resonance (MR) angiography and computed tomography (CT) angiography, performed without direct arterial injection and

without iodinated contrast agent injection for MR angiography, can be alternatives to classic arteriography, especially for distal and calcified lesions.

Neurologic Examination

To evaluate sensation ("sensation" in the PEDIS system), the Semmes-Weinstein 10-gram monofilament should be used. Loss of protective sensation in the affected foot is defined as absent light pressure sensation at two out of three sites on the plantar side of the foot (plantar aspect of hallux, first metatarsal, and fifth metatarsal), as described in the International Consensus on the Diabetic Foot [15]. The properly calibrated 10-gram monofilament is an objective and simple instrument used to screen the diabetic foot for loss of protective sensation and has shown to be a significant and independent predictor of likely lower extremity amputations in the diabetic population [50]. The PEDIS classification system considers the use of a 128-Hz tuning fork applied to the dorsum of the hallux at the base of the proximal phalanx to evaluate the presence or absence of vibration sensation. Although both tests have similar sensitivities for evaluating protective sensation loss, and combining modalities appears to increase specificity [51], we consider that, for practical purposes, the 128-Hz tuning fork should be reserved for clarifying equivocal results on the Semmes-Weinstein 10-gram monofilament test in DFI cases. The cause and severity gradation of protective sensory loss are difficult to evaluate and do not provide clinically useful information [15], consequently, they are not considered necessary.

Wound Evaluation

Wound evaluation ("extent/size" and "depth/tissue loss items" in the PEDIS system) includes the evaluation of the size and depth of the wound, both of which should be determined after debridement. The size could be evaluated using a precise technique (planimetry or grid technique), however, this is not always possible in clinical practice. Instead, wound size and depth can be estimated by multiplying the largest diameter by the perpendicular largest diameter [44]. Ulcer depth should be evaluated related to the structures involved. Ulcers are divided into lesions confined to the skin, those penetrating to the subcutaneous structures (fascia, muscle, or tendon) and those involving subsequent layers of the foot (bone and/or joint).

Infection

The diagnosis of infection ("infection" in the PEDIS system) is clinical, based on the presence of symptoms and signs of inflammation [15], and must always be confirmed and classified according to the depth of involvement. In the PEDIS grading system, three parameters are particularly relevant to clinical management and outcome: the involvement of the skin only (Grade 2), the involvement of deeper structures (Grade 3), and the patient's systemic inflammatory response (Grade 4). Further qualitative definitions of clinical interest should also be considered: cellulitis (infection of the subdermis), necrotizing cellulitis (infection-related tissue necrosis of the subdermis and dermis), necrotizing fasciitis (infection with involvement of the superficial fascia, presenting as sloughing of the skin and a violaceous color of the integument, without pus or abscess), wet gangrene (infection associated with blackish necrotic tissues), and osteomyelitis (infection of the bone). To definitively categorize the patient into one of these groups different diagnostic procedures are indicated. All patients should have a complete blood count with differential, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) testing and, ideally, procalcitonin (PCT) testing. However, caution must be exercised when interpreting laboratory tests, as no marker is sufficiently sensitive and specific to confirm the diagnosis of DFI and tests are often misleading, even in the case of severe lesions [19]. In these patients, the most sensible sign of infection is often recalcitrant hyperglycemia despite regular

anti-hyperglycemic regimens. In our clinical experience, CRP has proven to be a good test in the diagnosis and follow up of serious DFIs. The values of more specific inflammatory markers (i.e., combined CRP and procalcitonin) might also be of value in discriminating DFI and could help to ensure a more rational use of antibiotic agents [52]. Recognizing the insensibility of classical signs and laboratory tests for the diagnosis of DFI and that various factors suggest the presence of DFI in the absence of these classical signs [53], Lipsky et al. developed a DFI wound score that could also act as a reliable and useful tool for predicting clinical outcome [54].

Another problem is determining the presence of osteomyelitis **(Figure 5)**. The International Working Group on the Diabetic Foot has proposed consensus criteria [55] for diagnosing diabetic foot osteomyelitis **(Table 1)** that remain to be validated in a properly designed trial. Clinical signs (pus in bone at surgery, detached bone in ulcer, visible cancellous/cortical bone, or chronic inflamed wound) and laboratory signs (elevated ECR) are highly variable, and some patients may have no signs that suggest underlying bone infection [56]. A positive probe-to-bone test (i.e., when a sterile metal probe reveals a hard and gritty surface compatible with bone) in the presence of DFI appears to have a relatively variable positive predictive value, while a negative test in a low-risk patient markedly decreases the likelihood of osteomyelitis [57]. Plain radiographs should be the initial imaging study, because in established cases, they often show characteristic pathological findings (cortical erosion, periosteal reaction, and mixed lucency and sclerosis). However, they are relatively insensitive, particularly in the first two

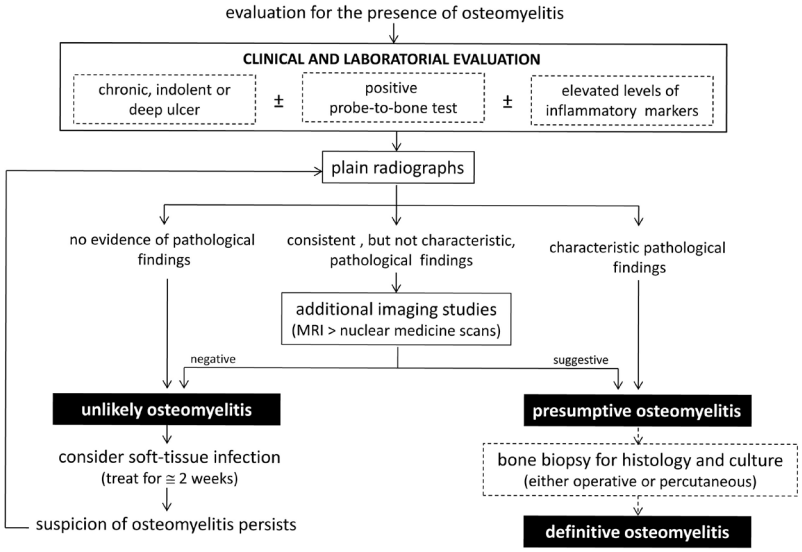

Figure 5: Evaluation for the presence of osteomyelitis. The suspicion of osteomyelitis may arise from clinical or laboratory signs. The initial imaging studies should be plain radiographs, as they often show characteristic pathological findings in established cases. However, because they are relatively insensitive in the first two weeks, additional imaging studies may be necessary. Magnetic resonance (MR) imaging, which has a very high sensitivity for bone and deep soft-tissue infections, is preferred to nuclear medicine scans. The gold standard for diagnosing osteomyelitis remains histopathology of a positive culture from a properly obtained bone specimen.

Foot Osteomyelitis	GRADE-0	GRADE-1
Definite	**Any 1 of the following:** – positive bone culture and histology – pus in bone at surgery – detached bone in ulcer – bony abscess on MRI	2 probable findings 4 possible findings 1 probable + 2 possible findings
Probable	**Any 1 of the following:** – visible cancellous bone – MRI findings highly indicative of osteomyelitis – positive bone culture or histology	2 possible findings
Possible	Any 1 of the following: – cortex erosion on X-ray – MRI findings compatible with osteomyelitis – positive probe-to-bone – visible cortical bone – ESR >70 mm/h – chronic inflamed wound	
Unlikely	Any 1 of the following: – normal MRI – normal bone scan – acute ulcer without inflammation – normal X-ray	

Table 1 - International Working Group on the Diabetic Foot Consensus Criteria for Diagnosing Diabetic Foot Osteomyelitis

weeks after infection when no changes are frequently found. Furthermore, they are nonspecific, because they do not permit the differential diagnosis of noninfectious neuro-osteoarthropathy [58].

To overcome this problem, many clinicians use technetium bone scans. These scans are more sensitive but rather nonspecific, especially in neuropathic patients [10]. Combining bone scans with other scintigraphic techniques, such as white blood cell scans (Indium-111 leukocyte scans or other variations), improves specificity, although these tests are rarely used because they are expensive and time consuming [59]. Studies [56] have shown that the best imaging test, when available, is MR imaging. MR imaging has a very high sensitivity for bone and deep soft-tissue infections. Despite its high cost, this imaging test has gained wide acceptance in the management of patients with DFI [60], as it can also be used for surgical planning. Newer techniques, such as positron emission tomography (PET) scans, appear promising, but their role is as yet undefined [61]. The criterion gold standard for diagnosing osteomyelitis is a characteristic histopathology (acute or chronic inflammatory cells, or necrosis) associated with a positive culture from a properly obtained bone specimen ideally obtained at the time of surgical debridement or by fluoroscopic- or computed tomography-guided percutaneous biopsy [55]. In comparison, culture results from associated soft tissue wounds or sinus tracts do not reliably correlate with those taken from bone [60].

In the absence of suspected osteomyelitis, bacteriological sampling, which must be done after mechanical debridement and cleansing of the wound with gauze soaked in sterile physiological saline, is indicated if a DFI ≥ Grade 2 is clinically confirmed. The best sampling technique remains a matter of debate. While tissue biopsy and fluid aspirate are considered the gold standard for diagnosing wound infection [28], such invasive tests are infrequently performed for superficial wounds or in many practice settings, such as outpatient clinics, due to concerns about enlarging the ulcer or inducing pain [28,62]. Superficial swabbing of the wound is discouraged, but swabbing the base of the ulcer is acceptable if it is the only possible option [11]. Independent of the sampling method, specimens must be placed in transport medium and be sent to the microbiology laboratory as quickly as possible. Assuming that there are no completely reliable microbiological methods to distinguish between pathogenic and nonpathogenic microorganisms at the present time, microbiologists and clinicians must collaborate closely to interpret the results. They must also take into account the collection conditions transport time, transport conditions, and the type of bacteria isolated. These procedures are summarized in **Figure 6**.

Other Diagnostic Procedures

Other diagnostic procedures may be indicated in the assessment of DFIs. However, it should be noted that many of these tests lack the ability to provide a definitive diagnosis, and clinical correlation is always required.

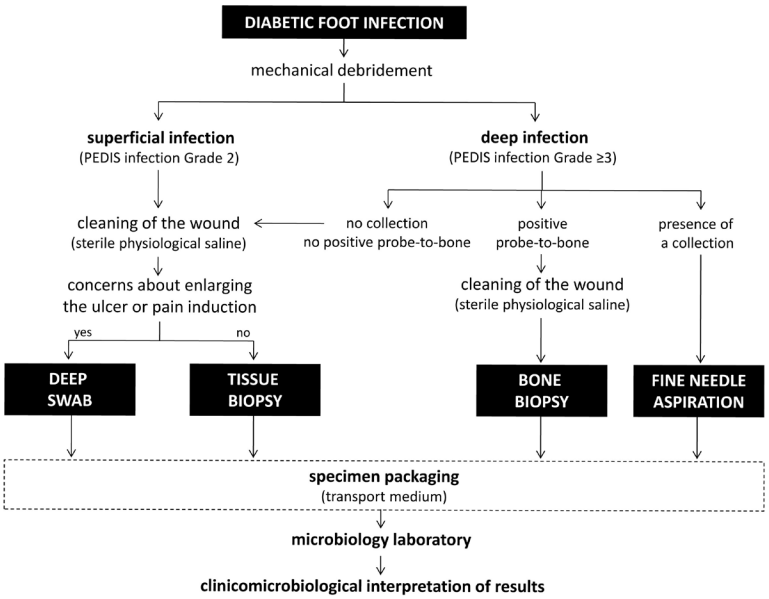

Figure 6: Choice of specimens to be performed as a function of the type of wound. Bacteriological sampling is indicated if a diabetic foot infection (DFI) corresponding to PEDIS Grades ≥2 has been clinically confirmed. Mechanical debridement and wound cleansing with gauze soaked in sterile physiological saline must precede sampling. Tissue biopsy and fluid aspirate are considered the gold standard for diagnosing wound infection, but deep swabbing of the wound is acceptable in special circumstances. Microbiologists and clinicians working in close collaboration must interpret the results, while taking into consideration the collection conditions, transport time, transport conditions, and the type of bacteria isolated.

Treatment

Although research continues to improve wound-healing modalities and show promising options for the future, the importance of prevention cannot be overemphasized. The clinically predictable progression from DFU to DFI, present in 85% of amputation cases [63], highlights the importance of implementing an integrated, standardized prevention and treatment protocol. In this disease-management program, prevention strategies should be delivered by family physicians and diabetologists using structured screening tools at defined intervals, with high-risk patients referred to multidisciplinary foot care teams for treatment. Even when using these well-defined interfaces, less than 20% of patients are referred to specialized diabetic foot clinics [64]. When a DFI patient presents to the care team, a multidisciplinary management strategy should be rapidly implemented **(Figure 7)**. As previously described, evidence suggests that this reduces the incidence of major amputation. The multidisciplinary team should include an endocrinologist/diabetologist, a surgeon with relevant expertise in managing DFI, a tissue viability nurse, and, ideally, a podiatrist. These professionals should also have access to other specialist services such as those provided by vascular surgeons and orthopedists [11].

When treating a DFI, the multidisciplinary team must consider the need for hospitalization, prioritize the treatment and drainage of any invasive infection, and perform limited debridement if necrosis is present. The assessment of vascular supply adequacy as well as a complete and appropriate vascular reconstruction follows. Further treatment should be based on the severity of the infection. Superficial ulcers without residual ischemia can usually be treated on an outpatient basis with repeated debridement, off-loading, and oral antibiotics. In other ulcers, formal debridement should be completed and, as accumulating evidence indicates, negative pressure wound therapy (NPWT) should be included in the treatment pathway [65].

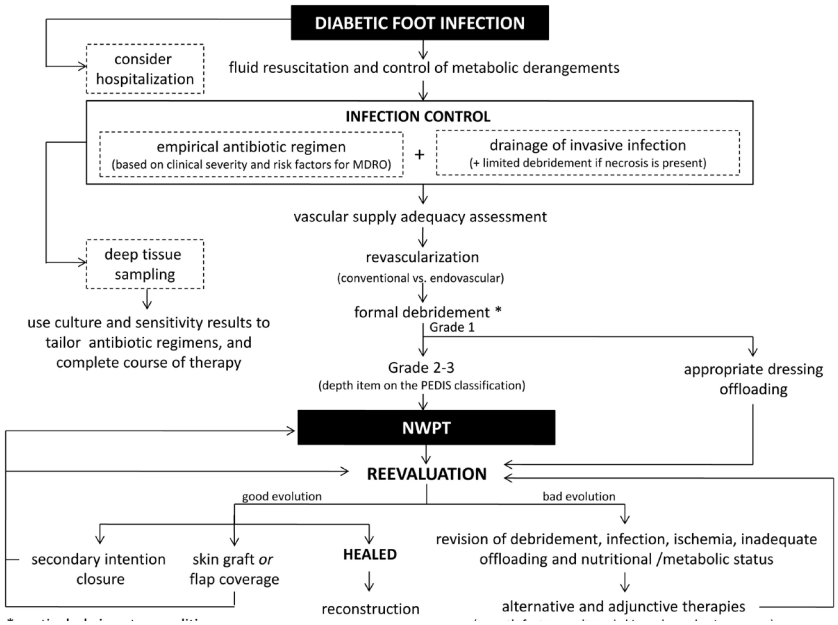

Figure 7: Management of diabetic foot infections (DFI). The multidisciplinary team must consider draining invasive infections, debriding necrosis, and promptly starting empirical antibiotic therapy, followed by complete and appropriate vascular reconstruction. Further treatment should be based on the severity of the infection. Superficial ulcers without residual ischemia can usually be treated on an outpatient basis. For other ulcers, a formal debridement should be conducted. Accumulating evidence also suggests that negative pressure wound therapy (NPWT) should be included in the treatment pathway. Assuming that debridement, infection, ischemia, offloading, and nutritional status are addressed, a wound that fails to improve within 2 to 4 weeks should prompt the clinician to consider alternative and adjunctive therapies. A biomechanically sound reconstruction, with or without amputation, should be part of the treatment plan to minimize the risk of recurrent ulceration.

When used after adequate debridement in a well-vascularized bed, NPWT prepares the wound for closure by secondary intention or skin graft. A wound that fails to improve within 2 to 4 weeks should prompt the clinician to consider alternative and adjunctive therapies (assuming appropriate attention to debridement, infection, ischemia, offloading, and nutritional status). A biomechanically sound surgical reconstruction, with or without amputation, must be considered part of the treatment plan to minimize the risk of recurrent ulceration.

Need for Hospitalization

Hospitalization is the first decision to make regarding patients with a DFI, and determining its necessity requires considering many aspects. Patients with severe infection (Grade 4), deep wounds, suspected bone and joint involvement, and severe ischemia (gangrene) should be hospitalized. They often may require surgical intervention (debridement, drainage, bone resection, or possibly urgent revascularization), fluid resuscitation, and metabolic derangement regulation through strict glycemic control (usually using insulin therapy). These are at least as important as selecting proper antibiotic therapy [10]. Other factors suggesting the need for hospitalization include metabolic instability (e.g., severe hypoglycemia or ketoacidosis), expected poor patient compliance, and the impossibility of outpatient care [10].

Infection Control: Empirical Antibiotic Therapy and Invasive Infection Drainage

Invasive infection drainage should be the first-line treatment for all ulcers, especially those associated with abscesses complicated by compartmental syndrome, extensive necrosis, or necrotizing cellulitis. Randomized clinical trials have shown that systemic antibiotics (including the most recently available agents) are of clinical value in DFI [10,66] and, as in the majority of infectious diseases, they must be provided as early as possible. However, as authoritative guidelines emphasize [10,11] and a recent systematic review confirms [67], no particular antimicrobial regimen has been shown to be superior to others in DFI treatment. The initial empirical antibiotic therapy in DFIs should aim to cover the most common pathogens and should be based on the known epidemiology of DFIs. They should subsequently be refined according to initial response and elements of the patient assessment [29]. The severity of infection is essential in determining the appropriate antibiotic regimen [10]. Patients with mild infections who have not previously received antibiotic therapy usually have an infection caused by only one or two species of bacteria [68], and an antibiotic regimen should almost always include an agent active against Staphylococcus aureus and Streptococcus spp. [29]. Long-standing or severe DFIs need extended coverage to include commonly isolated Gram-negative bacilli, Enterococcus spp., and anaerobes. When culture and sensitivity results are available, these should be considered to select a narrower-spectrum antibiotic therapy and complete the course of therapy. However, some important considerations should be taken into account (Figure 8).

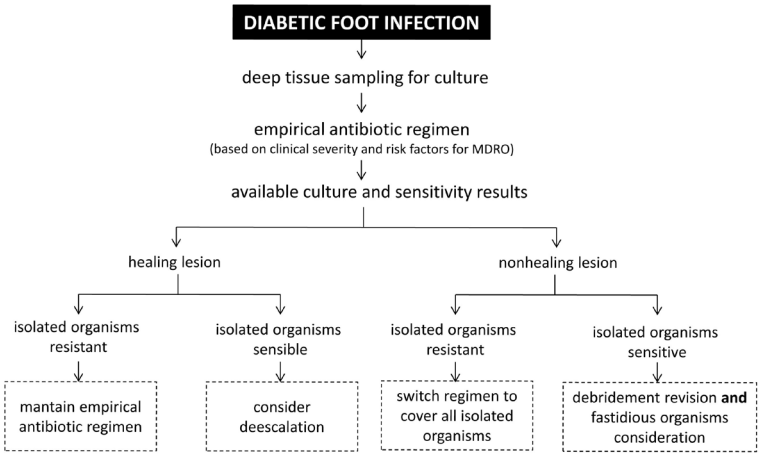

Figure 8: Empirical antibiotic regimen review. When culture and sensitivity results are available, these should be considered to select a narrower-spectrum antibiotic therapy and complete the course of therapy. If the lesion is healing and the patient is tolerating the empirical regimen, there may be no reason to change, even if some or all of the isolated organisms are resistant to the agents used. On the other hand, if the infection is not responding then the treatment should be changed to cover all of the isolated organisms. If the infection is worsening despite susceptibility of the isolated microorganisms, the fastidious organisms may have been missed on culture and a revised debridement should be conducted.

If the lesion is healing and the patient is tolerating the empirical regimen, then there may be no reason to change, even if some or all of the isolated organisms are resistant to the agents used. This is well illustrated by the Sidestep study [69], in which a favorable clinical response to ertapenem was noticed in patients in whom Enteroccus spp. and Pseudomonas aeruginosa were isolated despite the ertapenem resistance of the latter isolates. This may be due to either the disruption of microbial synergy or the differential importance of individual virulence potential. On the other hand, if the infection is not responding then treatment should be changed to cover all the isolated organisms. If the infection is worsening despite the isolated microorganisms' susceptibility, the possibility that fastidious organisms were missed on culture should be taken in account and a revision of debridement should be done.

Other factors that must be taken into account when selecting an antibiotic regimen are the route of administration (oral vs. antibiotics), the penetration of antibiotics into infected diabetic foot tissues, and the cost of treatment. The parenteral route should be used for severe infections, in the neuroischemic foot, when the agents used cannot be administered orally, or when the patient's state is incompatible with oral therapy. In all other cases, outpatient oral therapy is recommended, provided that regular medical follow-up can be ensured. The optimal duration of antibiotic therapy has not been clearly established, but it could be 1 to 2 weeks for simple forms and 2 to 4 weeks for moderate to severe forms of skin and soft tissue infections. Cost is also an important consideration, and antibiotic therapy should proceed as indicated by the clinical situation and severity of the infection, with the lowest cost. Understanding these basic principles behind choosing an antibiotic regimen is more important than knowing particular antibiotic regimens, and each hospital should have epidemiology-based antibiotic guidelines for DFI management. A final consideration is topical antimicrobial therapy. Although it's not currently advisable for most clinically infected chronic wounds, it may have a role in specific circumstances [70]. The application of any topical antibiotic should always be preceded by formal debridement and may be considered for a properly managed wound with subclinical infection that is failing to heal or to help in the removal of biofilms, which have been implicated in persistent infections [71].

The presence of bone infection substantially alters the approach to therapy, but there are no validated or well-accepted guidelines for treating diabetic foot osteomyelitis [29,60]. Classically, bone resection in chronic osteomyelitis was considered essential to a cure [60]. This routine, however, has recently been disputed [72] because radical surgical solutions (such as transmetatarsal amputations) may result in altered biomechanics, with a consequent higher risk of reulceration. Published nonrandomized case series on nonsurgical treatment with a prolonged (3 to 6 months) course of antibiotics have reported clinical success in 65% to 80% of cases [10,73]. While optimal therapy requires obtaining bone for culture, initial empirical therapy should virtually always cover Staphylococcus aureus, which causes most infections. The traditional recommendations of initial parenteral therapy may be outdated by the introduction of newer agents with excellent oral bioavailability [29]. Selected patients may benefit from implanted antibiotics (e.g., embedded in beads or cement), HBOT, or revascularization, whereas others may elect for long-term or intermittent antibiotic suppression [10].

Revascularization

In the case of critical ischemia, once the infection has been controlled, revascularization must be immediately considered. Ideally, revascularization should be done at the same time as the formal debridement procedure. In other cases, revascularization can be deferred and proposed secondarily, especially in cases of delayed healing. In these later cases, the criterion for revascularization should consider the patient's status

performance, the potential for cicatrization, the site of the lesions, and the quality of the arterial distal runoff. The current revascularization options for the DM patient include conventional open surgery and endovascular interventions, which are not mutually exclusive and are often combined. Open surgical techniques include endarterectomy for local lesions and peripheral bypass for long occlusions. For bypass surgery, a single segment of the greater saphenous vein is the best conduit, although acceptable results have been obtained with prosthetic grafts that do not cross the knee [74]. Long-term results are good, with 5-year secondary patency rates >70% and limb salvage rates of 75 to 85% [75]. Endovascular options include angioplasty, with or without stenting, and atherectomy (i.e., atherectomy with excimer laser or a plaque excision device). This treatment modality has a number of advantages over bypass surgery, particularly its low morbidity and mortality rate. However, the restenosis rate is relatively high, especially in below-the-knee procedures for which it is as high as 50% over a 5-year period [76]. Since the main goal for patients with DFI is to obtain sufficient perfusion to control the infection and save the limb, this is significant since this temporary improvement of perfusion can be sufficient enough to promote healing and avoid amputation. However, it takes up to 28 days after endovascular intervention for the new blood flow to have maximal effect at the wound's edge, whereas this time frame is reduced to 4 to 10 days after bypass surgery [77]. The results of recent studies [78] indicate that endovascular therapy might take a prominent place in the treatment of PAD, especially in patients with significant comorbidities, and this applies even more to patients with DFI. Antiplatelet therapy should begin preoperatively and continue after an endovascular or surgical procedure [25].

Formal Drainage and Debridement

Drainage and debridement (surgical, mechanical, sharp, etc.) are two different but complementary surgical procedures. Drainage is the incision of an area of tissue phlegmon or abscess. This surgical procedure is particularly important in deep infections of the plantar surface of the foot, where infection spreads through the tendon sheaths of the toe flexor muscles located in the compartment between the superficial fascia and the arch of the foot. This compartment serves as a non-expandable box, resulting in a compartment syndrome that leads to ischemia and tissue necrosis. Under these circumstances, the drainage must open the plantar fascia. Drainage of collected deep DFIs is urgent, in the authors' opinion. The debridement process involves physically excising necrotic material and debris until normal tissue appears, thus enabling wound healing and removing a reservoir of potential pathogens [79]. Mechanical debridement, as elegantly demonstrated by Wolcott et al. [80], also opens a time-dependent antimicrobial therapeutic window. Members of the multidisciplinary team should be the only ones to perform debridement. They should implement the technique that best matches their specialist expertise and clinical experience, the patient's preference, and the site of the ulcer [11]. Efforts should be made to only remove dead tissue, while preserving as much other viable tissue as possible. Sharp debridement with a scalpel, scissors, or tissue nippers is the conventional procedure; however, to minimize damage to normal tissue, which sometimes occurs with normal surgical sharp debridement techniques, alternative debridement with a hydrosurgical water knife may be used [81]. In all of the procedures, serial thin slices of tissue should be removed until normal tissue appears.

The presence of clotted vessels, stringy fascia, or tendon indicates that the tissue is not viable and should be removed and shaved down to shiny hard tendon or fascia. Soft, grey bone is necrotic and should be resected to reveal clean, hard bone with punctuated bleeding at the surface. Odor is an excellent indicator of adequate debridement, and if the wound is odorless post-debridement the clinician can feel comfortable that the wound has been adequately debrided [64].

Wet-to-dry dressings, hydrotherapy, biotherapy, and other topical debriding agents provide alternative options to surgical debridement. Unfortunately, they are less definitive and controllable, require prolonged and repeated applications, and delay the application of other therapies [82]. Debridement should be performed as soon as possible, bearing in mind that in neuroischemic ulcers, formal debridement other than drainage of infection should only be performed after or during revascularization procedures.

Negative Pressure Wound Therapy (NPWT)

NPWT includes a family of devices consisting of specialized dressings, including adhesive drape and open-cell foam, cut to fill the wound defect and capable of transmitting constant or intermittent pressure throughout the wound using a feedback control mechanism. NPWT has proven its effectiveness in various diabetic foot wounds in several randomized, controlled studies [83-85]; however, most of these studies have not addressed the preoperative infectious status, and few have addressed the use of NPWT in DFI. Be that as it may, recent physiopathological and clinical evidence justifies its use as a useful adjunct to the management of DFIs [65].

Excessive physiopathological exudate in DFIs can be detrimental because it contains an imbalance of matrix metalloproteases (MMPs) and their inhibitors (TIMPs). NPWT increases the diffusion gradients, which facilitates the removal of excess interstitial fluid and infectious materials and improves blood flow as well as consequent metabolic waste evacuation. Whether NPWT actually reduces the bacterial load is debatable, however, its clinical effectiveness in DFIs has been demonstrated. The original animal study by Morykwas et al. [86] and the clinical study by Argenta et al. [87] showed that NPWT use resulted in enhanced granulation tissue formation with improved bacterial clearance compared with control dressings. In other studies [88], NPWT reduced the bacterial count to 104 to 106 per gram of tissue within 4 to 5 days. However, in a retrospective study, Weed et al. [89] showed that despite the clear beneficial effects of NPWT, bacterial colonization increased significantly within the range of 104 to 106 per gram of tissue during prolonged therapy. This was also evident in a study by Moues et al. [90], in which NPWT was compared with conventional moist dressing therapy and did not significantly decrease the total bacterial load. There were qualitative differences, however, with nonfermentative Gram-negative bacilli showing a significant reduction and Staphylococcus aureus showing a significant increase. This suggests that bacterial burden reduction may occur within the first 4 to 5 days, making subsequent therapy effective in decreasing wound size. This was also seen in the first large prospective and randomized study of NPWT use in the treatment of complex clean diabetic foot wounds [83]. NPWT was effective (i.e., it showed a higher and faster healing rate with lower major amputation rates) at the cost of a higher but non-statistically significant infection rate.

Several subsequent studies have demonstrated NPWT's effectiveness in DFIs, particularly to treat osteomyelitis and soft tissue infections [65,91-93], when used in conjunction with adequate debridement and appropriate antibiotics. A new strategy to amplify the bioburden control using a modified NPWT system with an infusion port to intermittently instill antimicrobial agents has been developed [94], but it has not been properly investigated in the clinical setting. In DFIs, there are no contraindications to NPWT, but special care should be taken to cover exposed blood vessels, prosthesis, or bone with natural tissues or several layers of fine-meshed, non-adherent synthetic material [64]. Finally, but importantly, a number of studies [65,95,96] suggest that adding NPWT as part of a wound management strategy results in shortened hospital stays and a higher percentage of limb salvage, with consequent decreased overall medical costs.

Based on the presented evidence, we propose NPWT utilization as defined by Kim et al. [65]. NPWT should be applied immediately after the revascularization procedure and formal debridement, while the patient is in the operating room. For the first 2 or 3 days, a continuous suction mode of 125 mmHg should be used and the dressing should be changed every 12 to 24 hours, at which time the wound should be carefully evaluated for any residual necrotic material and subsequent debridement should be performed, if appropriate [64,97]. Once the infection is controlled and the wound is stable, the suction mode should be changed to an intermittent cycle of 5 minutes on and 2 minutes off. The dressings should then be changed every 24 to 48 hours, as this has been shown to increase blood flow and improve granulation [86].

Off-loading and Non-NPWT Wound Dressings

No intervention is likely to be successful if the wound is not protected against external trauma; therefore, complete and permanent off-loading of the wound must be ensured as strictly as possible. Many types of devices can off-load the infected wound, but it is important to choose one that permits easy inspection. In inpatients, either bed rest or wheelchair use (keeping the affected leg horizontal) is preferred [10,11]. It is beyond the scope of this paper to analyze all available wound dressings. As there is insufficient evidence to recommend a specific wound dressing or any type of wound healing agent for DFIs, we agree with the National Institute of Health and Clinical Excellence (NICE) guidelines [11] that advise the multidisciplinary team to consider their clinical assessment of the wound, the patient's preference, clinical circumstances, and acquisition cost.

Reevaluation and Further Treatment

If infection or wound necrosis worsens (bad wound evolution) during NPWT, a review of debridement, infection, ischemia, and nutritional/metabolic status should be performed. In this case, NPWT discontinuation should be considered. If the wound fails to improve despite repeated surgical interventions, alternative and adjunctive therapies should be considered (e.g., growth factors and hyperbaric oxygen therapy [HBOT]). According to a meta-analysis [98], G-CSF treatment might reduce the duration of hospitalization, the risk of lower-limb amputation, and other infection-related invasive intervention, but it does not appear to have a significant effect on the duration of intravenous antibiotic treatment, the resolution of infection, or the rate of wound healing. Systemic HBOT has also been proposed, as infection and ischemia are considered the two main indications for this procedure. Its use was recently analyzed in a systematic review [99] of five RCTs in which it was associated with a reduction in major amputations, but not in minor amputations or time to heal. We agree with Lipsky et al. [10] that only additional randomized clinical trials can establish protocols for using these expensive and limited resources to treat DFIs. If these treatments fail or are not considered, amputation remains the only option in cases of severe infection, especially in the neuroischemic foot. The amputation level depends on the vascular status and should preserve heel weight-bearing with prosthesis. Major (leg or thigh) amputations should be exceptional, occurring only in cases of uncontrolled life-threatening infection. If healthy and well-vascularized granulation tissue is observed (good evolution), depending upon the location and size of the wound, either a skin graft (for large skin defects on non-weight bearing surfaces) may be conducted, or secondary intention healing (for wounds on weight bearing surfaces) may be induced. A skin graft is an effective way to close a chronic ulcer, but it requires a healthy and well-vascularized granulation bed to survive. To avoid infection while ensuring the adherence of the graft to the underlying bed and avoiding seroma or hematoma development, NPWT may still be necessary after grafting [100]. After the wound has healed, a biomechanically sound foot reconstruction must be completed in the presence of deformity to prevent the recurrence of foot ulceration.

CONCLUSION

DM is a global epidemic and the diabetic foot is one of its most frequent and serious complications, resulting in high social and economic costs. According to the International Working Group on the Diabetic Foot, a leg is lost to DM somewhere in the world every 30 seconds, with infection accounting for 50% of these cases [4].

The five-year mortality rate for diabetes-related wounds and amputations is 68%, only surpassed by lung and pancreatic cancer mortality rates [101]. We hope this hospital-based framework for diagnosing and treating DFIs will help improve the hospital management of DFIs and ultimately the prognosis of these patients.

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